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Research Article

The Effect of Dietary Vitamin E on the Arginase Activity in the Females of Freshwater Crayfish (*Astacus leptodactylus*, Esch. 1823)

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Abstract: The effects of different levels of dietary vitamin E on the arginase activity of the hepatopancreas, gills and muscle of freshwater crayfish (*Astacus leptodactylus*) were investigated. The control diet contained approximately 37.41% crude protein on a dry-weight basis and 3.25 kcal g^{-1} gross energy. The diet ingredients contributed 65.83 mg kg^{-1} vitamin E to the control diet. Levels of 34.17 mg kg^{-1}, 84.17 mg kg^{-1} and 134.17 mg kg^{-1} vitamin E (DL-α-tocopherol acetate) were added to the control diet to supply 100 mg kg^{-1} in diet 1, 150 mg kg^{-1} in diet 2 and 200 mg kg^{-1} in diet 3, respectively. The study was carried out in triplicate for 272 days.

The results showed that arginase activities in the hepatopancreas and gills were not significantly affected by the different levels of dietary vitamin E. Vitamin E levels higher than 100 mg kg^{-1} caused an about 2-fold reduction in the activity of muscle arginase in the females with stage-1 juveniles in comparison with the control ($P < 0.01$). In the ovigerous crayfish in comparison with the control, arginase activity in the muscle was slightly increased (but not significantly) by 100 mg kg^{-1} vitamin E levels, and slightly decreased by 200 mg kg^{-1} vitamin E in diets.

The presence of vitamin E higher than 150 mg kg^{-1} in the ovigerous crayfish and 100 mg kg^{-1} in the females with stage-1 juveniles in diets may negatively affect the connective tissue formation by decreasing the muscle arginase activity.

Key Words: Arginase, freshwater crayfish (*Astacus leptodactylus*), vitamin E

Dişi Kerevitlerin (*Astacus leptodactylus*, Esch. 1823) Arjinaz Aktivitesi Üzerine Vitamin E'nin Etkisi

Özet: Kerevit (*Astacus leptodactylus*) rasyonuna farklı miktarlarda vitamin E ilavesinin hepatopankreas, solungaç ve kas arjinaz aktivitesi üzerine etkisi araştırılmıştır. Bu amaçla 3,25 kcal g^{-1} toplam enerji ve % 37,41 oranında ham protein içeren bir kontrol rasyonu hazırlanmıştır. Rasyon öğeleri kontrol rasyonuna 65,83 mg kg^{-1} vitamin E katkısı sağlamıştır. Bu rasyona 34,17 mg kg^{-1}, 84,17 mg kg^{-1}, 134,17 mg kg^{-1} miktarlarında vitamin E (DL-α-tocopherol acetat) ilave edilerek sırasıyla 1, 2, 3 nolu deneme rasyonları oluşturulmuştur. Bu deneme 3 tekrar olarak 272 gün sürdürülmüştür.

Sonuçlar, rasyonda farklı miktarlarda bulunan vitamin E'nin hepatopankreas ve solungaç arjinaz aktivitesini etkilemediğini göstermiştir. Yavrulu dönemdeki kerevitlerin rasyonunda 100 mg kg^{-1}'dan fazla vitamin E bulunması, kas arjinaz aktivitesinin yaklaşık iki misli azalmasına sebep olmuştur ($P < 0,01$). Yumurtalı dönemdeki kerevitlerde kas arjinaz aktivitesi, rasyon 100 mg kg^{-1} vitamin E içerdiğinde istatistik olarak önemli olmayan bir artma, 200 mg kg^{-1} vitamin E içerdiğinde ise azalma göstermiştir.

Rasyonda yumurtalı kerevitlerde 150 mg kg^{-1}, yavrulu kerevitlerde ise 100 mg kg^{-1}'dan fazla vitamin E bulunması kaslarda arjinaz aktivitesini azaltarak bağ doku oluşumunu olumsuz yönde etkileyebilir.

Anahtar Sözcükler: Arjinaz, kerevit (*Astacus leptodactylus*), vitamin E

Introduction

Organisms are classified as ammoniotelic, uricotelic, or ureotelic depending on the end product of nitrogenous metabolism. Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) catalyses the hydrolysis of L-arginine to form L-ornithine and urea in the final reaction of the urea cycle. In ureotelic species, arginase activity is most abundant in the liver, where the urea cycle is most active (1).

Arginase is also found in organs and organisms that do not synthesise urea (2,3). Although the crayfish is an ammoniotelic organism and does not contain an active urea cycle, it has also an active arginase enzyme (4-7).

Arginase activities have been measured in the hepatopancreas of 15 species of marine invertebrates and the highest arginase activities have been found in the Crustacea (5). The presence of arginase in tissues of organisms using other pathways for disposing of nitrogen and lacking a complete urea cycle has been explained as a consequence of deletion or repression of the genes controlling the production of urea cycle enzymes (2).

The determined amounts of vitamin E have been supplemented to diets to increase pleopodal egg number in crayfish, to modulate the antioxidant defence system and to provide optimum growth in prawn (8-10). Park and Tappel (11) reported that there was a relationship between vitamin E and arginase, and that rats fed a vitamin E supplemented diet had lower liver arginase activity than those of rats fed a vitamin E deficient diet.

This experiment was designed to investigate whether the different levels of dietary vitamin E may affect the arginase activity of freshwater crayfish (*Astacus leptodactylus,* Esch. 1823).

Table 1. Composition and proximate analysis of the control diet (modified after ref. 12).

Ingredient	Percent of dry weight
Fish (anchovy) meal	35.78
Soybean meal	38.64
Wheat flour	19.30
Sunflower oil	4.00
Dicalcium phosphate	1.00
Sodium phosphate	0.40
Avilamycine[1]	0.10
Antioxidant[2]	0.10
Vitamin premix[3]	0.50
Mineral premix[4]	0.18
Proximate composition	
Crude protein	37.41
Crude fat	7.60
Crude fibre	4.00
Crude ash	14.95
Nitrogen free extract	29.56
Moisture	6.48
Gross energy (kcal/g)	3.25
Protein/Energy (mg/kcal)	115.23

(1) Kavilamycine

(2) Antioxidant (mg/kg dry diet): butylated hydroxytoluene 12.5.

(3) Vitamin premix (IU or mg/kg): vitamin A 2,000,000 IU, vitamin D_3 200,000 IU, vitamin E 20,000 IU, vitamin K 3000 mg, vitamin B_1 1000 mg, vitamin B_2 3000 mg, Niacin 30,000 mg, Calcium D-Pantothenate 10,000 mg, vitamin B_6 2000 mg, vitamin B_{12} 4 mg, Folic Acid 600 mg, D-Biotin 200 mg, Choline Chloride 100,000 mg and vitamin C 60,000 mg.

(4) Mineral premix (mg/kg dry diet): Mn 80, Fe 35, Zn 50, Cu 5, I 2, Co 0,4, Se 0,15.

Materials and Methods

This study was carried out between October 1 2001 and June 29 2002 (272 days) at the crayfish reproduction unit of Fisheries Faculty of Fırat University, Elazığ, Turkey. The crayfish used in the present study were obtained from the Keban Dam Lake population of *A. leptodactylus*.

The practical control diet used in this study (Table 1) was modified after Reigh et al. (12). The control diet was formulated to contain approximately 37.41% crude protein on a dry-weight basis and 3.25 kcal g^{-1} gross energy. Levels of dietary vitamin E (supplied as DL-α-tocopherol acetate) were 100, 150, 200 mg kg^{-1} diet for diets 1, 2 and 3, respectively. No vitamin E was added to the control diet, except that supplied by the vitamin premix and feed ingredients. DL-α-tocopherol acetate was donated by Roche (İstanbul). The ingredients for each diet were thoroughly mixed, before adding water, in a commercial food mixer, cold-pelleted by forcing through 3-mm holes using a laboratory pellet mill, air-dried at 55 °C for up to 24 h, and then stored in a refrigerator at 4 °C until further use.

The vitamin E contents of the control diet, diet 1, diet 2 and diet 3 were 65.83 mg kg^{-1}, 100 mg kg^{-1}, 150 mg kg^{-1} and 200 mg kg^{-1}, respectively, on a dry-weight basis. Vitamin E levels of the control and experimental diets were analysed by high performance liquid chromatography (13). The crude protein content was analysed by Kjeldahl's method. The gross energy was calculated based on physiological fuel values of 9 kcal g^{-1} for lipid and 4 kcal g^{-1} for protein and carbohydrate. The dry matter was determined after the sample was dried at 105 °C for 6 h. The ash content was determined after 24 h at 550 °C in the furnace. The lipid was analysed by an ether extraction method (14).

During the trial, mean dissolved oxygen was 7.5 ± 1.0 mg l^{-1}. Mean ammonia, iron and copper content were less than 0.001 mg l^{-1} (for each parameter). Mean calcium was 39.2 ± 1.6 mg l^{-1}. Mean alkalinity was 217.3 ± 2.5 mg $CaCO_3$ l^{-1}. Mean hardness was 34 ± 4 FSº, and mean pH was 7.8 ± 0.3 (15). Mean water temperature was 9.6 ± 5.3 °C; supplemental water flow was 0.5 l sec^{-1} for each tank. Dissolved oxygen, pH and water temperature were measured daily. Ammonia, iron, copper, alkalinity, hardness, calcium and water flow were measured twice a week. The crayfish were exposed to a natural photoperiod.

The experiment was carried out in 3 replicates for each dietary treatment. To determine the effect of vitamin E on the arginase activity, 12 females with eggs (ovigerous) and 12 females with stage-1 juveniles were used for each replicate. The hepatopancreas, gills and muscle tissue were excised. The tissues were weighed and homogenised with 10 volumes of 10mM Tris-HCl buffer (pH 7.4) in a glass Potter Elvehjem homogeniser in an ice bath. The homogenates were centrifuged at 20,000 xg for 10 min at 4 °C. The supernatants were used for the arginase assay.

Arginase activity was measured spectrophotometrically in optimised conditions for crayfish (6) by the thiosemicarbazide diacetylmonoxime urea (TDMU) method (16). One unit of arginase activity was expressed as the amount of enzyme catalysing the formation of 1 µmol of urea h^{-1} at 37 °C. The results are given as units mg^{-1} of protein.

Protein was measured as described by Lowry et al. (17) using bovine serum albumin as standard.

The results were expressed as mean ± SEM. Analysis of variance (ANOVA) followed by Duncan's test was used to determine whether there were significant differences among the groups. Differences were considered as significant when P values were less than 0.05.

Results

The results showed that arginase activities in the hepatopancreas and gills were not significantly affected by the different levels of dietary vitamin E (Tables 2 and 3). Vitamin E levels higher than 100 mg kg^{-1} caused an about 2-fold reduction in activity of muscle arginase in the females with stage-1 juveniles in comparison with the control ($P < 0.01$) (Table 3). In the ovigerous crayfish in comparison with the control, arginase activity in the muscle was slightly increased but not significantly by 100 mg kg^{-1} vitamin E levels in the diet, and slightly decreased when 200 mg kg^{-1} vitamin E was present in the diet (Table 2).

Table 2. The effects of different levels of dietary vitamin E on the arginase activity (units mg^{-1} of protein) in the ovigerous crayfish.

Vitamin E	Control				P
	65.83 mg kg^{-1}	100 mg kg^{-1}	150 mg kg^{-1}	200 mg kg^{-1}	
Hepatopancreas	0.27 ± 0.14	0.18 ± 0.01	0.33 ± 0.13	0.39 ± 0.13	-
Muscle	1.75 ± 0.35^{ab}	2.39 ± 0.27^{a}	1.75 ± 0.23^{ab}	1.05 ± 0.09^{b}	$P < 0.01$
Gills	0.74 ± 0.15	0.66 ± 0.09	0.92 ± 0.12	0.78 ± 0.17	-

- : $P > 0.05$

± values: standard error of the means

Values with different superscripts within the same line were statistically significant ($P < 0.05$).

Table 3. The effects of different levels of dietary vitamin E on arginase activity (units mg^{-1} of protein) in the females with stage-1 juveniles.

Vitamin E	Control				P
	65.83 mg kg^{-1}	100 mg kg^{-1}	150 mg kg^{-1}	200 mg kg^{-1}	
Hepatopancreas	0.93 ± 0.13	0.76 ± 0.13	0.64 ± 0.13	0.89 ± 0.15	-
Muscle	0.46 ± 0.08^{a}	0.51 ± 0.10^{a}	0.20 ± 0.04^{b}	0.21 ± 0.04^{b}	$P < 0.01$
Gills	2.93 ± 0.70	2.25 ± 0.71	2.92 ± 0.73	2.02 ± 0.61	-

- : $P > 0.05$

± values: standard error of the means

Values with different superscripts within the same line were statistically significant ($P < 0.05$).

Discussion

In aquatic vertebrates it was established that feeding, hormonal, seasonal and environmental (temperature, moisture, salinity, water deprivation etc.) conditions alter arginase activity (18-23). Food conversion, and hence growth, depend greatly on food intake (24) and in both teleosts and ureotelics feeding is an important factor affecting arginase activity (18,19,22,23,25,26).

In various studies, vitamin E has been added to diets in order to increase pleopodal egg number in crayfish, to modulate the antioxidant defence system and to provide optimum growth in prawn (8-10). Park and Tappel (11) reported that rats fed a vitamin E supplemented diet for 40 days had lower liver arginase activity than those fed a vitamin E deficient diet. The dietary vitamin E administration significantly reduced the liver arginase activity induced by high doses of prednisolone in rats (27). Furthermore, in the present study, it was observed that the presence of vitamin E higher than 150 mg kg^{-1} in the ovigerous crayfish and 100 mg kg^{-1} in the females with stage-1 juveniles in diets caused a reduction in the muscle arginase activity. Moreover, Harlioglu and Barim (28) reported that the presence of 100 mg kg^{-1} vitamin E in the diet caused significantly bigger and more pleopodal eggs, and stage-1 juveniles in freshwater crayfish, *Astacus leptodactylus.*

Carbamoyl phosphate synthetase and ornithine transcarbamoylase from urea cycle enzymes have been shown to be absent in crustaceans (4). In addition, crustaceans do not only have an active arginase enzyme but also the enzymic capacity to convert ornithine (the second reaction product of arginine hydrolysis) to proline (4,7). Proline is a fundamental structural element of collagen (connective tissue). Animals such as the earthworm, starfish and mussel evolving to use increased amounts of collagen synthesise proline from the ornithine moiety of arginine (4,29). In the light of this study, it can be concluded that the presence of vitamin E higher than 150 mg kg^{-1} in ovigerous crayfish and 100 mg kg^{-1} in females with stage-1 juveniles in diets may negatively affect connective tissue formation by decreasing muscle arginase activity.

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